ORCA Bioreactor™

Operator's Manual

HARVARD APPARATUS
REGENERATIVE TECHNOLOGY

Harvard Apparatus Regenerative Technology, 84 October Hill Drive, Holliston, MA 01746 USA
www.harvardbioscience.com ¨ 508.893.8999¨regenmedicine@harvardapparatus.com

Contents

Symbols	4
Overview	5
Chapter 1—Introduction	6
Chapter 2—Getting Started	7
2.1 Safety Requirements	7
2.2 Equipment Components	8
2.3 Heater Reservoir	9
2.4 Pumps	10
2.5 Chambers	11
Chapter 3—Assembly Instructions	
3.1 Lung System	12-15
3.2 Heart	16
3.3 Aortic Valve System	17
3.4 Hollow Organ System	18
3.5 Pump Head Assembly / Disassembly	19
3.6 Accessories	19
Chapter 4—Operating Instructions	
4.1 Sterilization	20
4.2 Reservoir Port Sizing	21
4.3 Controller Software	22-29
4.4 Decellularization	30
4.5 Recellularization	31
Chapter 5—Care & Maintenance	32
5.1 Parts List	33
Appendix A: Polestar Instructions	34-52
Appendix B: Frequently Asked Questions	53-55

Disclaimer:

Use of the ORCA Bioreactor™ should be conducted by a trained and manufacturer qualified representative. Harvard Apparatus Regenerative Technology does not warrant unauthorized use of this product; Harvard Apparatus does not warrant that the operation of this product will be uninterrupted or error-free and makes no claim of warranty or condition.

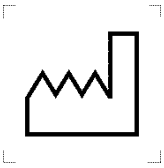
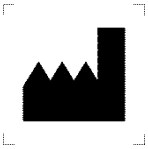
Harvard Apparatus reserves the right to change the instructions for use and any related products at any time without any prior notice and is not liable for any damages arising out of any change and/or alteration of the contents or product.

This product is for RESEARCH USE ONLY.

Copyright © 2013, Harvard Apparatus. All rights reserved.
ORCA Bioreactor™ is a trademark of Harvard Apparatus.
Harvard Apparatus, a division of Harvard Bioscience, owns the intellectual property rights to the ORCA Bioreactor. This material may not be reproduced, displayed, modified, or distributed without the expressed prior written permission of the copyright holder.

U.S., international, and foreign patent applications are pending.

Symbols Used

Symbol	Definition
	Date of Manufacture
	Serial Number
	Catalogue Number
	Warning: This action will have a direct impact on the patient
	Caution: This action will have an impact on the product or operator
	Manufacturer
CE	This device complies with Directive 2006/95/EC relating to electrical equipment designed for use within certain voltage limits; this device also complies with Directive 2004/108/EC relating to electromagnetic compatibility.

Overview

Harvard Apparatus Regenerative Technology partners with leading global scientists to provide specialized solutions.

The company is uniquely positioned to develop advanced instrumentation to accelerate regenerative medicine, tissue engineering and cell therapy experimentation. From the beginning, in 1901, Harvard Bioscience companies worked closely with leading global researchers to produce products with the highest levels of performance, quality and support necessary for the new challenges of your life science research.

We look forward to working with you to develop new tools to assist you in solving the new challenges of regenerative medicine from the lab bench to the patient. There are thousands of publications, in regenerative medicine to stem cell research, utilizing Harvard Apparatus products, but we are now introducing some newly developed products: one for regenerative organ generation and one for small volume cell delivery into organs. These products will serve researchers and physicians to accelerate both research and utilization of that research in patients.

Harvard Apparatus Regenerative Technology provides:

- Physiological bio-sensors and transducers to measure biological variables to better understand animal to cell physiology: mechanically, chemically, electronically
- Physiological data acquisition systems with advanced electronics, physiological data manipulation algorithms to measure, monitor, control and understand complex physiological events
- Physiological macro to nano fluidics infusion pumps
- Animal to cell electrophysiological monitoring systems
- Organ to cell perfusion bath and chambers to mimic physiological conditions to study drug, nutrient, gene and cell therapies
- Live cell imaging and perfusion chambers to better understand the affect of shear force, drugs, nutrition on cells and tissues
- Cell engineering tools from electroporation to pneumatic injectors

Chapter 1: Introduction

The ORCA Bioreactor is the first system designed to meet the needs of the modern regenerative medicine research scientist. The system is able to address the needs of both the decellularization and recellularization processes of various organs. **Physiological conditions** can be mimicked through control algorithms that regulate flow rates, profiles, and pressures. Critical readings can be taken from both inside the organ as well as in the support environment through the use of **sensors**. The **Concurrent Method Development software** logs all modifications and operator entries along with the result that was measured with the system sensors, allowing a complete review of an experiment and direct translation into a research method. A **method storage system** makes it easy to reproduce methods and conditions across multiple experiments. The **image capture software** allows for images to be captured in real time from cameras monitoring visible, IR, UV, and fluorescence; other systems such as ultrasound / ECHO are supported.

1.1 Warning and Caution Statements

The use of a **WARNING** statement in this User Manual alerts you to a potential safety hazard. Failure to observe a warning may result in a serious injury to the user or patient.

The use of a **CAUTION** statement in this User Manual alerts you to where special care is necessary for the safe and effective use of the product. Failure to observe a caution may result in minor injury to the user or damage to the product or other property.

1.2 Intended Use

The ORCA Bioreactor is a system used for the purposes of monitoring and studying an isolated organ; it is intended for research use only.

WARNING:

Use of this device in non-research settings must be conducted under local Regulatory requirements; consult your local Regulatory Authority.

Chapter 2: Getting Started

The following conditions must be met prior to using the In-Breath Bioreactor:

2.1 General Safety Requirements

WARNING:

The ORCA Bioreactor should only be used by qualified personnel who have been trained by the manufacturer or other Authorized Representative. Unauthorized use of this device is not recommended.

WARNING:

To prevent contamination, aseptic procedures must be followed and personal protective equipment must be worn at all times when handling and using the Bioreactor.

WARNING:

Wherever blood products are used, Universal Precautions must be followed.

Facility Requirements

Assure that the facility is able to provide a clean, safe, and suitable area for aseptic cell processing. It is recommend that all manipulations of the unit once sterile are performed in a biological safety cabinet of at least BCS Level 2

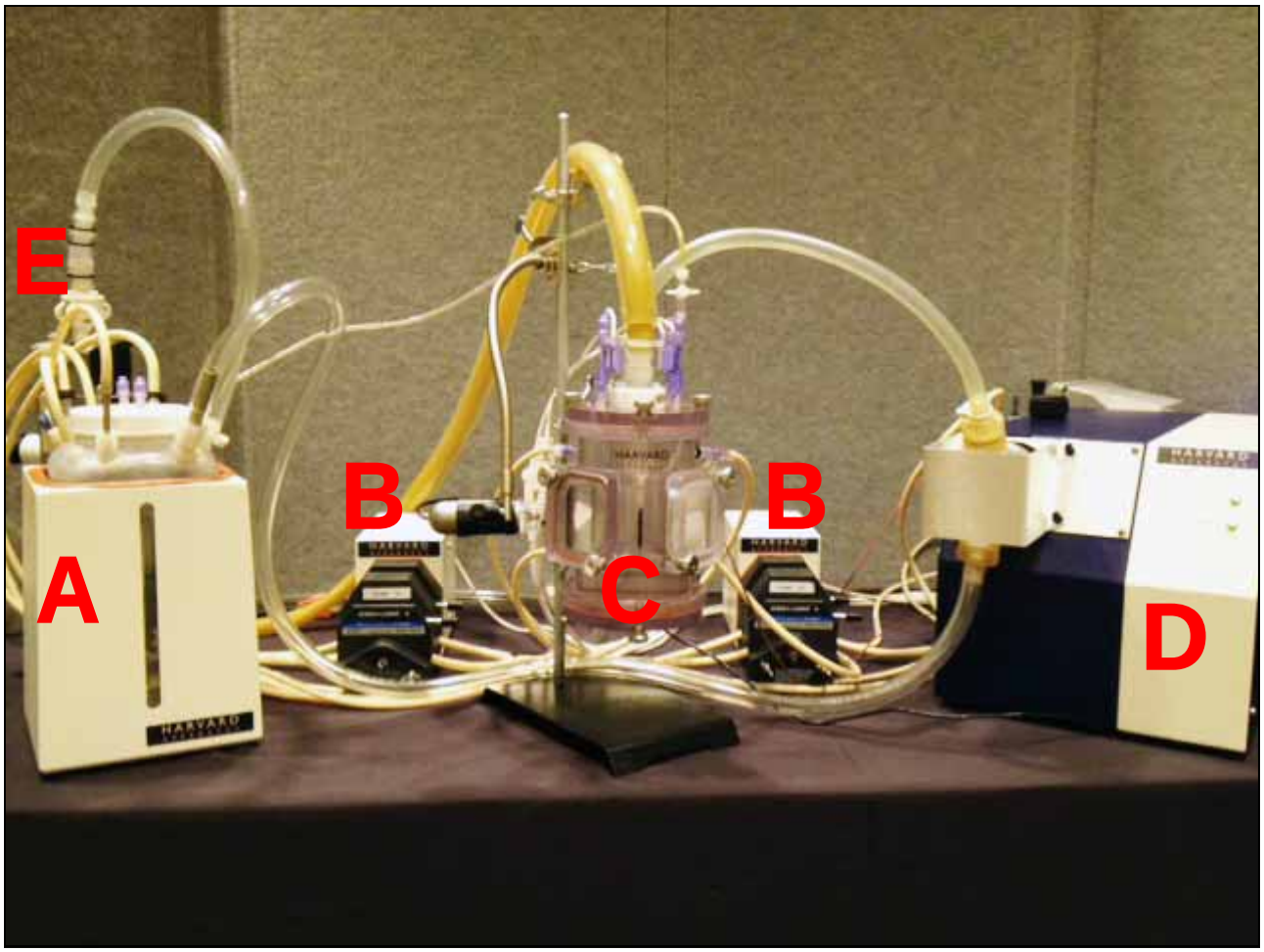
WARNING:

Failure to provide a means to conduct aseptic cell processing may result in harmful contamination.

2.2 Equipment Components

1) Store the ORCA Bioreactor in a cool, dry place, free from dust and other potential contaminants until ready to use.
2) Assure that the ORCA Bioreactor package was received completely and without damage; if the package arrived as damaged contact your local technical support group.
3) Ensure that all parts the ORCA Bioreactor package and its peripheral devices are in good condition. Do not use damaged parts.

Overview of Main ORCA Bioreactor Components



A) Heater Reservoir & Temperature Control System
B) Peristaltic Pumps
C) Organ Chamber
D) Pulsatile Pump
E) Oxygenator

Heater Reservoir

The heater is used to maintain temperature throughout the system to **emulate physiological conditions**.

The reservoir holds a volume of **0.5L to 4L** of media.

The reservoir has **built-in ports** for interaction with the oxygenator and the organ chamber, as well as a port for a 12” temperature monitoring probe.

There are two different sizes of bottles (large and small flow) that have different size ports to accommodate a wide variety of tubing and flows.

Pumps

Pumps are selected according to the organ and protocol requirements. The ORCA controller along with your pumps of choice address the needs of the **decellularization** and **recellularization** processes of various organs. All types of pumps may be controlled, including peristaltic, pulsatile and syringe.

A maximum of four pumps are used simultaneously on a system. The ORCA controller may be configured to control either four peristaltic pumps or three peristaltic pump heads and a pulsatile blood pump. Both configurations typically include **two** single channel dual head peristaltic pumps. A 4th pump may be added to the four unit controller to serve as a spare unit. Pump head configurations may be altered at any time to meet the most demanding protocols.

Selection of the pulsatile pump normally occurs when pulsatile flow rates in excess of 2L/min are required. This selection must be made at the time of purchase. If the “four peristaltic pump controller” is chosen, the fourth pump may be added at any time. A fourth peristaltic pump adds flexibility to accommodate elaborate protocols but is not required and can be added at a later time.

These configurations can be used to accommodate protocols that require a perfusion pump, ventilation pump and a pump that will provide flow to the oxygenation system and a gas monitoring system. Exterior medium feed and waste bags can also be accessed using channels on the double head double channel pump.

Pumps—Peristaltic

Peristaltic pump heads are available in **single** and **dual channel** versions.

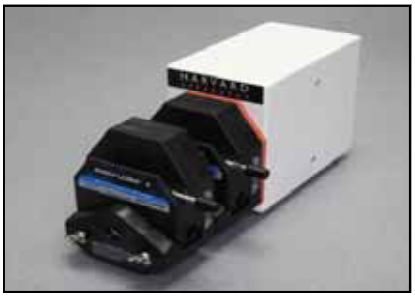
Multiple heads may be used on each peristaltic pump to provide flexibility in flow range as well as the number of channels required for the most challenging protocols.

High Capacity: Up to 1500mL/min

Mid Capacity: Up to 500mL/min

Single Channel (high capacity)

Single Head
(881001)



Dual Offset Head
(881003)

Dual Channel (mid capacity)

Single Head
(881002)

Dual Head [4 channel]
(881004)

Single & Dual Channel
(1 high capacity, 2 mid capacity)

Dual Head [3 channel]
(881005)

Pumps—Pulsatile

The pulsatile pump is used in some large animal systems to emulate ventricular action of the heart. It allows for minimal hemolysis and is ideal for moving emulsions, suspensions, and blood.

Chambers

Solid Organ Chambers

Large Solid Organ Chamber 14” (880420)
(holds approx. 32.8L)

Intermediate Solid Organ Chamber 10” (880429)
(holds approx. 11.6L)

Small Solid Organ Chamber 4.5” (880431)
(holds approx. 1.4L)

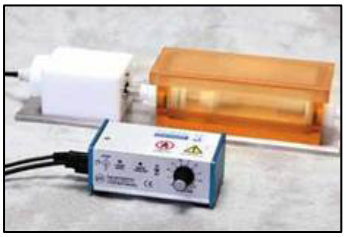
Solid organ chambers are autoclavable and are suitable for multiple species and sizes of organs. Tubing sets can be changed to accommodate the wide range of flow rates.

The layout of the chamber and use of clear materials allows for easy visibility of the organ being studied. Chamber orientation can be adjusted depending on the decellularization and cellularization procedures.

Chamber access ports are built in for removal and addition of media. Numerous sampling ports are included, and the built-in windows allow for manual access to an organ.

Hollow Organ Chambers

(880403)



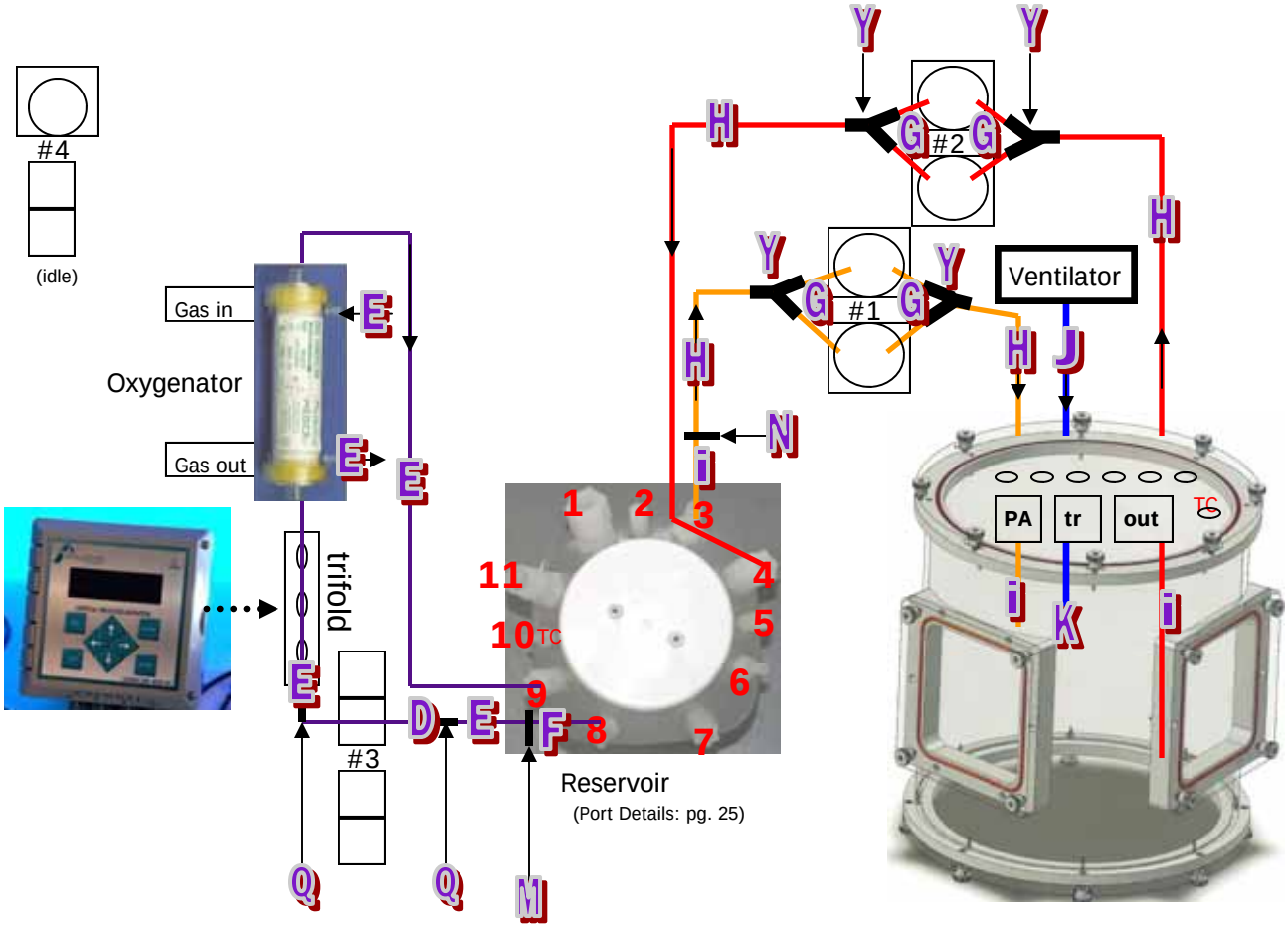
The rotating double chamber is specifically designed for cell seeding and culturing both surfaces of a tubular matrix. The **intraluminal** and **extraluminal** flow paths may be connected or maintained separately. The hollow organ chambers allow seeding and culturing of different cells types on either side of the tubular structure, providing homogeneity. The design allows for enhanced oxygenation and mass transport between the medium and cells.

Large and small animal versions are available.

Chapter 3: Assembly Instructions

3.1 Lung System

Note: The following is a diagram representing a configuration for the bioreactor for a *large animal lung system (**without cannulation** of the pulmonary vein)*. This diagram is not a replacement for your laboratory's protocols; be sure to follow your individual experimental procedures. Your individual laboratory's setup may differ from what is depicted below.



Key:

- Perfusion pathway into organ
- Oxygenation/Polestar pathway
- “Sipper” tube
- Ventilator pathway

Y Barb

Clamp

TC Thermocouple

Pumps

Pump #	Head Config	Typical Function
1	Dual offset	Perfusion into organ
2	Dual offset	Empty chamber to reservoir
3	4 channel	Oxygenation and analytics pathway
4	3 channel	Provides flexibility for the most challenging protocols

Tubing

Sizing	PharMed Opaque, flexible	Tygon Clear, flexible	Teflon Translucent, more rigid
1/8”	A **L/S #16** (0.12”, 1.6mm ID) 881105	B **1/8” ID** 721259	C **1/8” OD**
1/4”	D **L/S #17** (0.25”, 6.4mm ID) 881100	E **1/4” ID** 721256	F **1/4” OD** (30345)
3/8”	G **L/S #18** (0.31”, 7.9mm ID) 881101	H **3/8” ID** 721264	I **3/8” OD** (30346)
1/2”	—	J **1/2” ID** 721268	K **1/2” OD** (30347)

Clamps

L	1/8” clamp (xxxxxx)
M	1/4” clamp (721670)
N	3/8” clamp (721671)
O	1/2” clamp (xxxxxx)

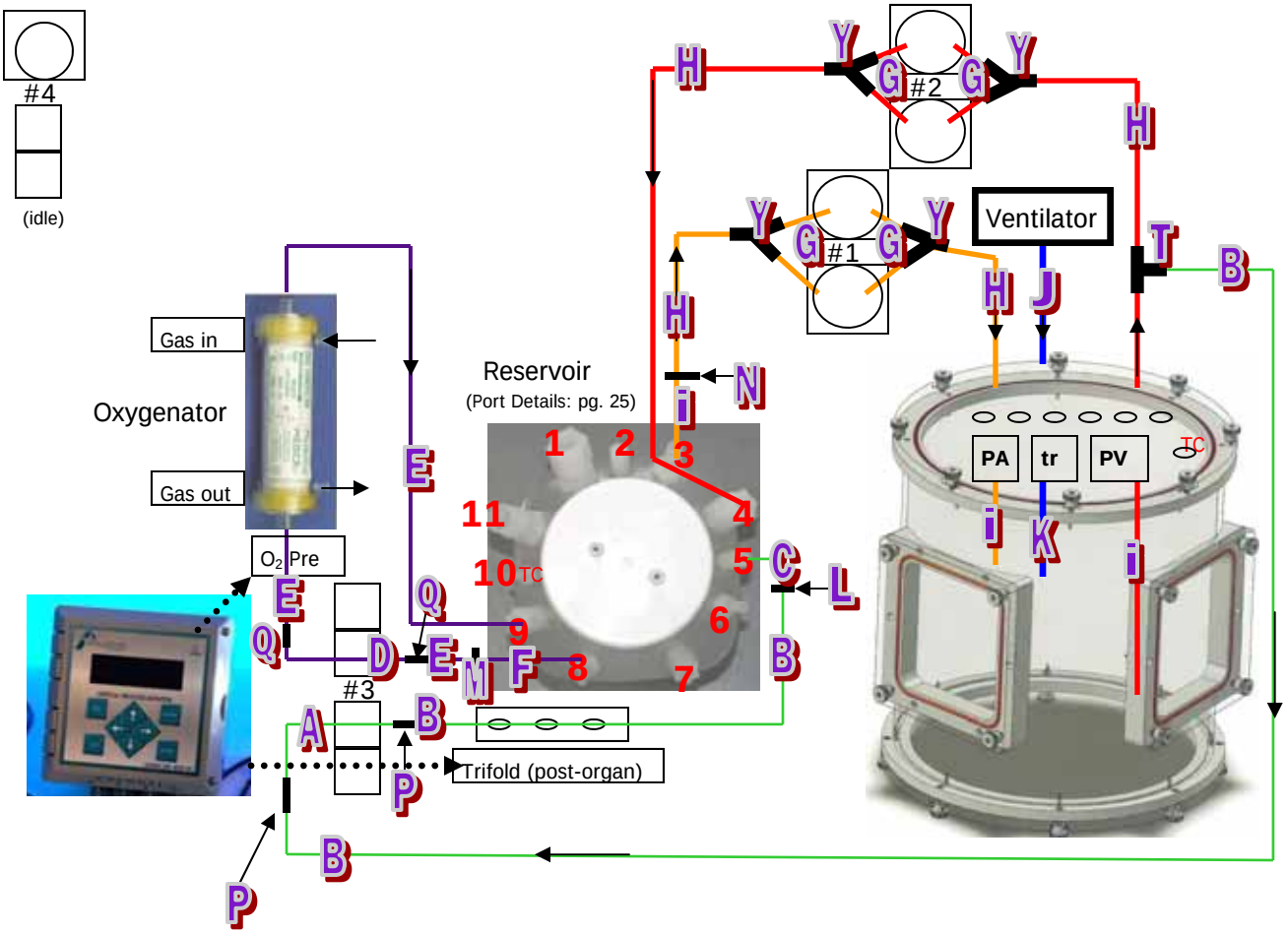
Connectors

P	1/8” connector (xxxxxx)
Q	1/4” connector (xxxxxx)
R	3/8” connector (721577)
S	1/2” connector (xxxxxx)

Y Barbs

Y	3/8” Y barb (xxxxxx)

Note: The following is a diagram representing a configuration for the bioreactor for a *large animal lung system (**with cannulation** of the pulmonary vein)*. This diagram is not a replacement for your laboratory's protocols; be sure to follow your individual experimental procedures. Your individual laboratory's setup may differ from what is depicted below.



Key:

- Perfusion pathway into organ
- Oxygenation/Polestar pathway
- “Sipper” tube
- Ventilator pathway
- Polestar transducer pathway
- Y Barb
- T Barb
- Clamp
- TC Thermocouple

Pumps

Pump #	Head Config	Typical Function
1	Dual offset	Perfusion into organ
2	Dual offset	Empty chamber to reservoir
3	4 channel	Oxygenation and analytics pathway
4	3 channel	Provides flexibility for the most challenging protocols

Tubing

Sizing	PharMed Opaque, flexible	Tygon Clear, flexible	Teflon Translucent, more rigid
1/8”	A **L/S #16** (0.12”, 1.6mm ID) 881105	B **1/8” ID** 721259	C **1/8” OD**
1/4”	D **L/S #17** (0.25”, 6.4mm ID) 881100	E **1/4” ID** 721256	F **1/4” OD** (30345)
3/8”	G **L/S #18** (0.31”, 7.9mm ID) 881101	H **3/8” ID** 721264	I **3/8” OD** (30346)
1/2”	—	J **1/2” ID** 721268	K **1/2” OD** (30347)

Clamps

L	1/8” clamp (xxxxxx)
M	1/4” clamp (721670)
N	3/8” clamp (721671)
O	1/2” clamp (xxxxxx)

Connectors

P	1/8” connector (xxxxxx)
Q	1/4” connector (xxxxxx)
R	3/8” connector (721577)
S	1/2” connector (xxxxxx)

Y Barbs

Y	3/8” Y barb (xxxxxx)

T Barbs

T	3/8” to 1/8” T barb (xxxxxx)

3.2 Heart System

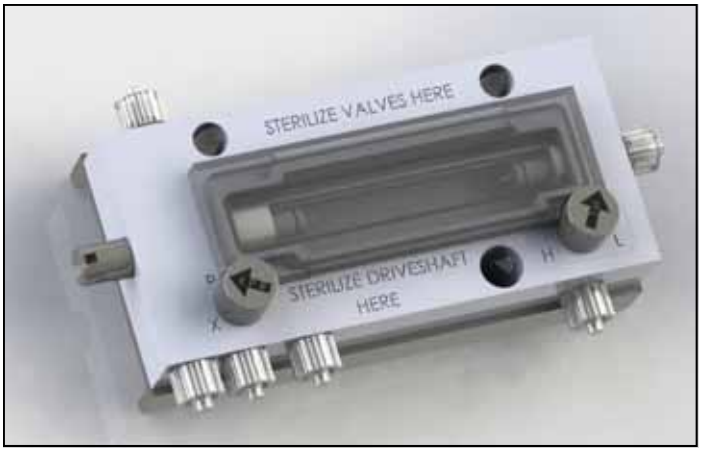
Note: The following is a diagram representing a configuration for the bioreactor for a *large animal heart system*. Your individual laboratory's setup may differ from what is depicted below.

3.3 Aortic Valve System

Note: The following is a diagram representing a configuration for the bioreactor for a *large animal aortic valve system*. Your individual laboratory's setup may differ from what is depicted below.

3.4 Hollow Organ System

Please refer to the Hollow Organ Bioreactor manual for information on assembly and operation of trachea / esophagus systems.



3.6 Accessories

Dino-Lite Cameras

Refer to the Dino-Lite manual for instructions.

PoleStar Gas Monitoring System

For regenerative medicine applications, please see **Appendix A** for information regarding setup and calibration.

For full manufacturer instructions, please refer to the PoleStar manual at **http://polestartech.com/support/pdf/dsp4000-user-manual.pdf**.

Chapter 4: Operating Instructions

Prior to Starting

Assure that all the requirements identified in Chapter 2 have been successfully met.

Personnel using the ORCA Bioreactor should read through this procedure in its entirety prior to using the device.

Personnel should train on all processes and equipment prior to operating the ORCA Bioreactor.

WARNING:

Failure to follow aseptic techniques and failure to train on all processes and procedures prior to using the In-Breath Bioreactor may result in critical delays, contamination, and other harmful events

4.1 Sterilization

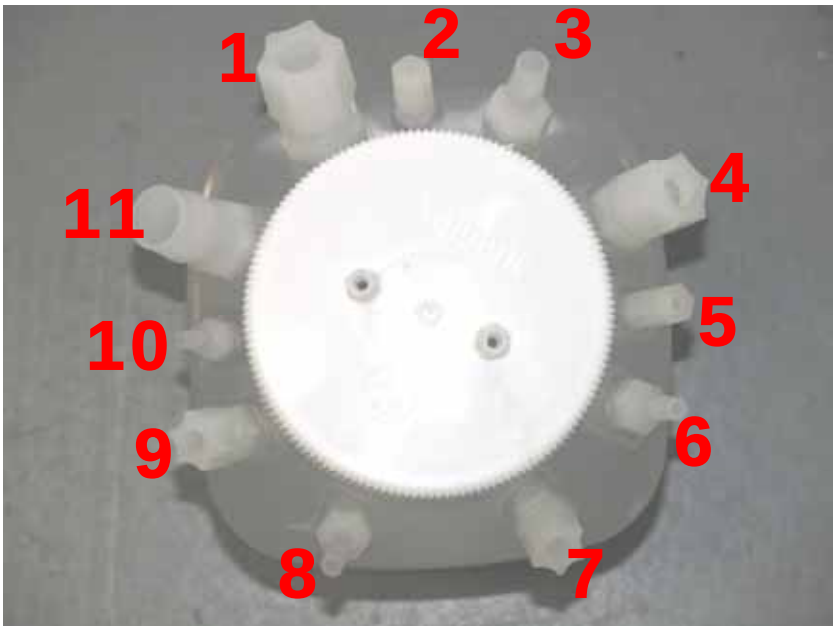
Sterilization can be performed using EtO or by autoclaving. **It is strongly recommended that all fittings be loosened prior to sterilization procedure.** One possible procedure would involve assembling the tubing set as it is to be used in the bioreactor and placing it into a sterilization bag to be autoclaved; the chamber could then be sterilized in a separate sterilization bag. Be sure to follow your laboratory's individual sterilization protocols.

Pharmed tubing may be used throughout the system. As a cost saving measure, or to have tubing that is transparent, Tygon tubing may be used (see illustrations). If the flow path is autoclaved has Tygon tubing, check all barb connections. In many cases Tygon tubing will become soft and may slide off the barb after autoclaving when pressure is applied. If the tubing is soft on the barb, it is recommended that the tubing that was on the barb is cut and discarded and that the fresh tubing is slid onto the barb. Ty-wraps may also be used on barb fittings.

4.2 Reservoir Bottle Configuration & Typical Usages

Large Animal

Note: This diagram depicts a typical usage for each of the reservoir bottle ports in a large animal system. Be sure to follow your laboratory's individual protocols.



1) 3/4" tube; Pulsatile for trachea

2) 1/8" tube; Polestar transducer

3) 3/8" rigid tube; PA on lung or pressure control for valve peristaltic

4) 3/8" tube; PA on lung or pressure control for valve peristaltic

5) 1/8" rigid tube; Polestar transducer

6) 1/4" rigid tube; chamber circulation

7) 1/4" tube; chamber circulation

8) 1/4" rigid tube; oxygenator circulation

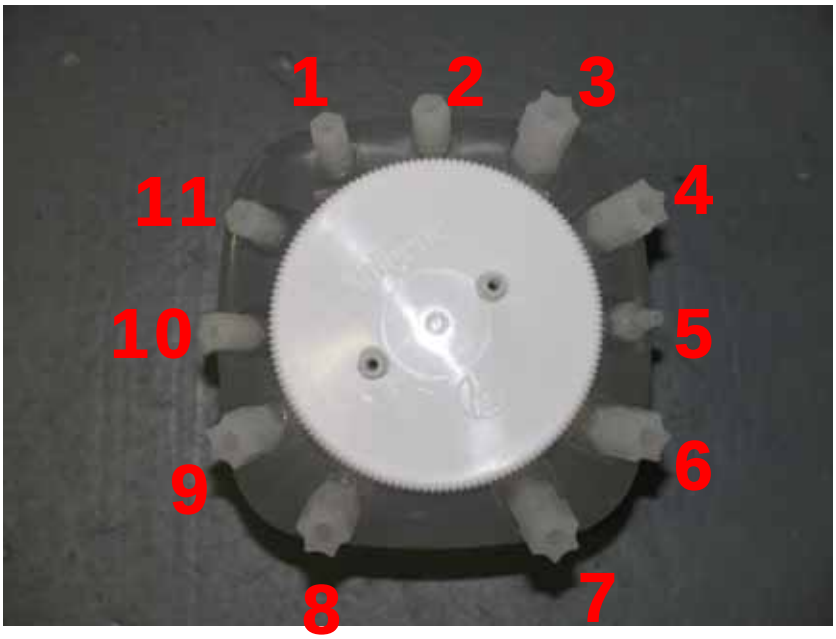
9) 1/4" tube; oxygenator circulation

10) 1/8"; thermocouple

11) 1/2" tube; Pulsatile for trachea

Small Animal

Note: This diagram depicts typical sizing & usage for each of the reservoir bottle ports in a small animal system. Be sure to follow your laboratory's individual protocols.



1) 1/8" tube

2) 1/8" rigid tube; Polestar transducer

3) 1/4" rigid tube; PA on lung or pressure control for valve peristaltic

4) 1/4" rigid tube; PA on lung or pressure control for valve peristaltic

5) 1/8" tube; Polestar transducer

6) 1/4" rigid tube; chamber circulation

7) 1/4" rigid tube; chamber circulation

8) 1/4" rigid tube; oxygenator circulation

9) 1/4" rigid tube; oxygenator circulation

10) 1/8"; thermocouple

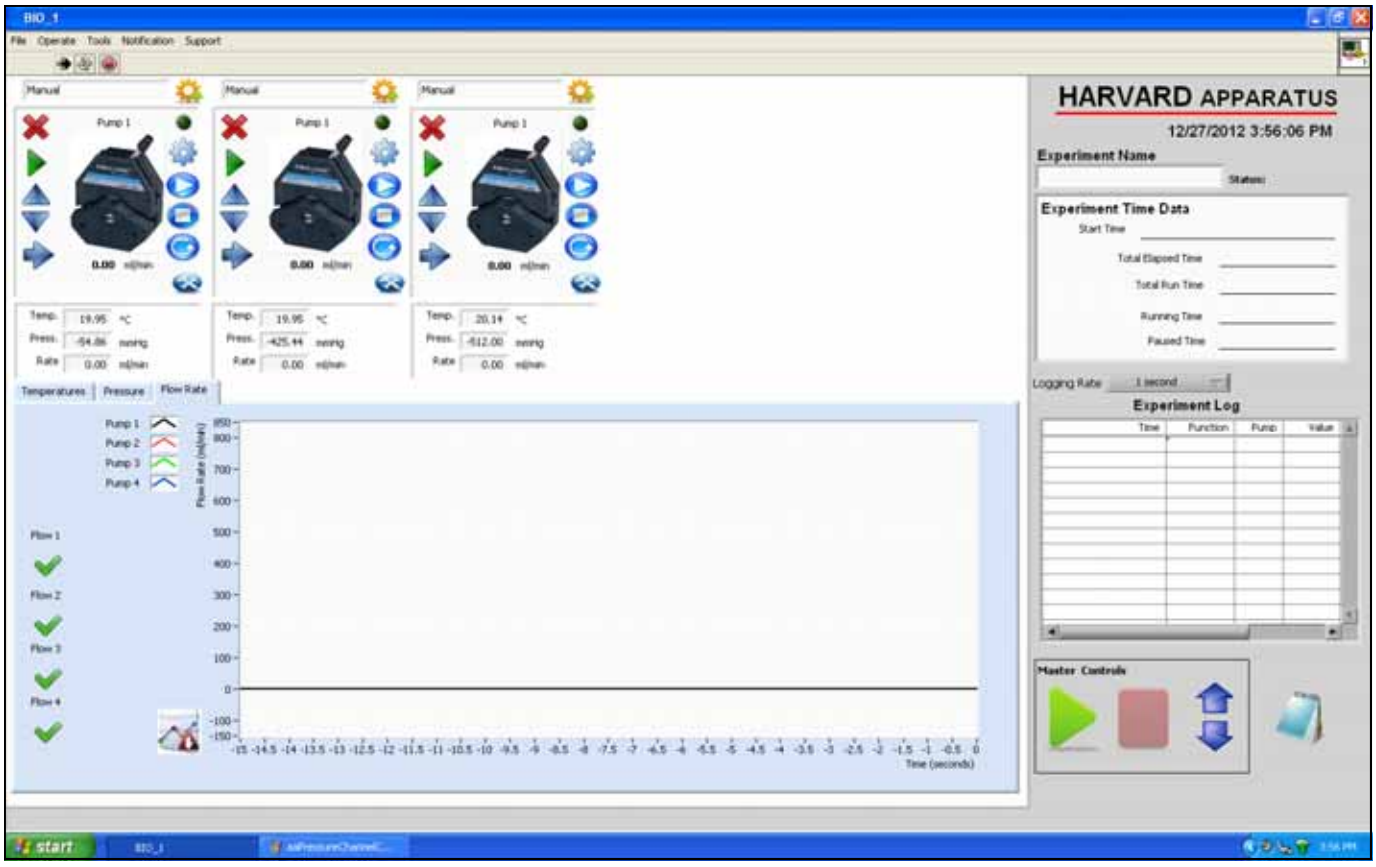
11) 1/8" rigid tube

4.3 Harvard Apparatus Pump Software

Operation Instructions

This pump operation software allows for control of flow rate and gives temperature, pressure, and flow rate readouts over time.

The user can control a maximum of four pumps at once; both pulsatile and peristaltic pumps can be utilized.



The Main Screen

Enable/Disable Pump
This primes a pump for use or deactivates a pump when not in use.

Start/Stop Run
Starts or stops the corresponding pump.

Flow Rate Control
These up/down arrows manually adjust the flow rate while the run takes place. The flow rate can also be altered by clicking on "**x.xx ml/min**" just below the pump icon and entering a value.

Flow Direction
Changes the direction that the pump rotates; a right arrow indicates clockwise.

Create or Select Step
Use this to create or select a Step. See "Step Creation" on pg.24 for additional info.

Green Light
Glows when pump is on.

Step Mode
See "Step Mode" on pg.22 for additional info.

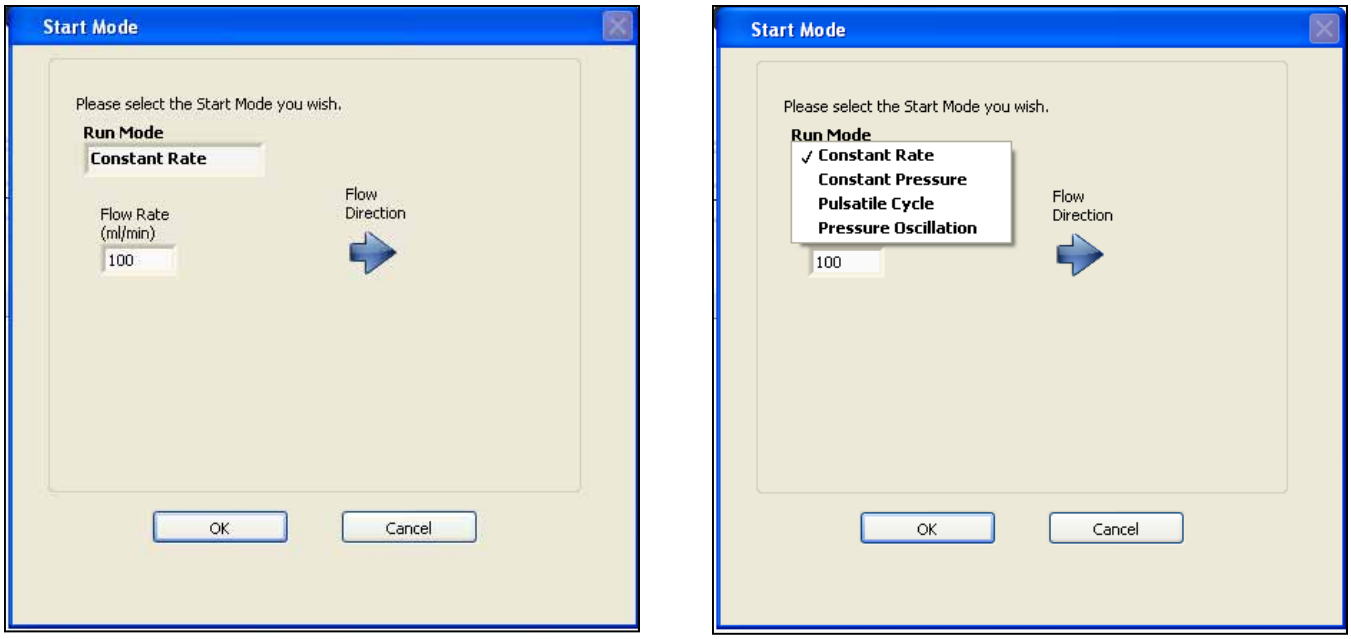
Start Mode
See "Start Mode" on pg.22 for additional info.

Stop Mode
See "Stop Mode" on pg.23 for additional info.

Repeat Mode
See "Repeat Mode" on pg.23 for additional info.

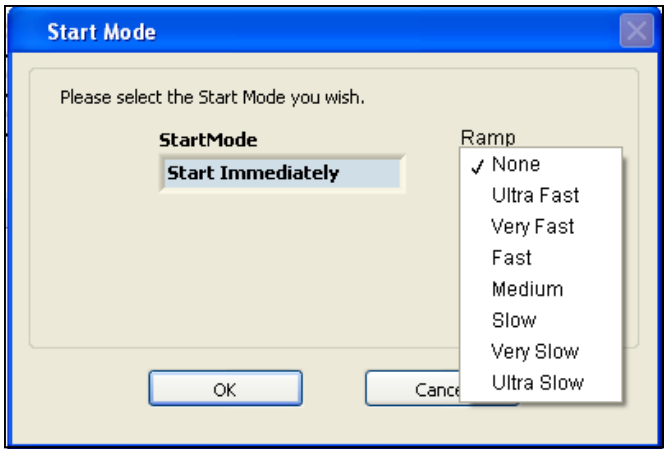
Pump Selector
Choose from peristaltic, syringe, pulsed blood, or ventilator modes.

Step Mode



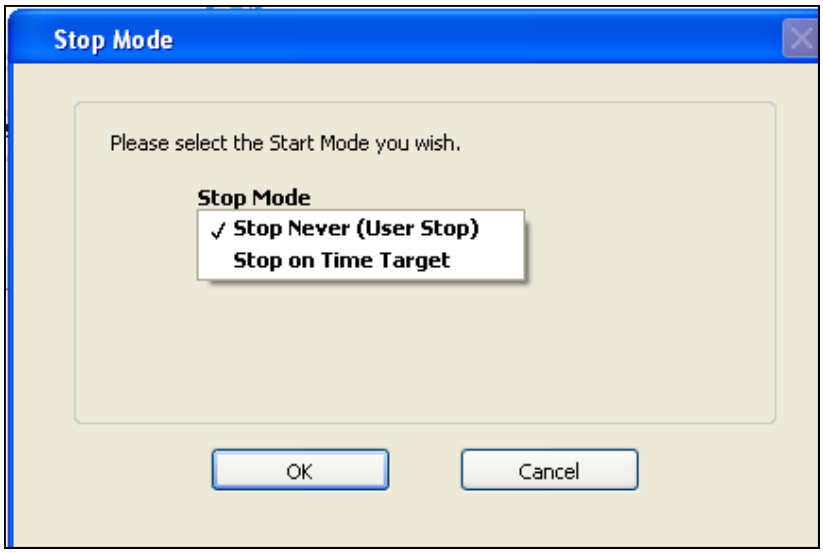
On this screen, **Run Mode, Flow Rate, and Flow Direction** can be controlled. For the **Run Mode** parameter, the pump can be set for **Constant Rate, Constant Pressure, Pulsatile Cycle,** or **Pressure Oscillation**.

Start Mode



This screen allows the user to choose the **Ramp** during start-up of the pump, giving a range of options from **ultra slow** to **ultra fast**. Selecting **no ramp** is an option. The user can also toggle the **Start Mode** between starting immediately and starting with a delay.

Stop Mode

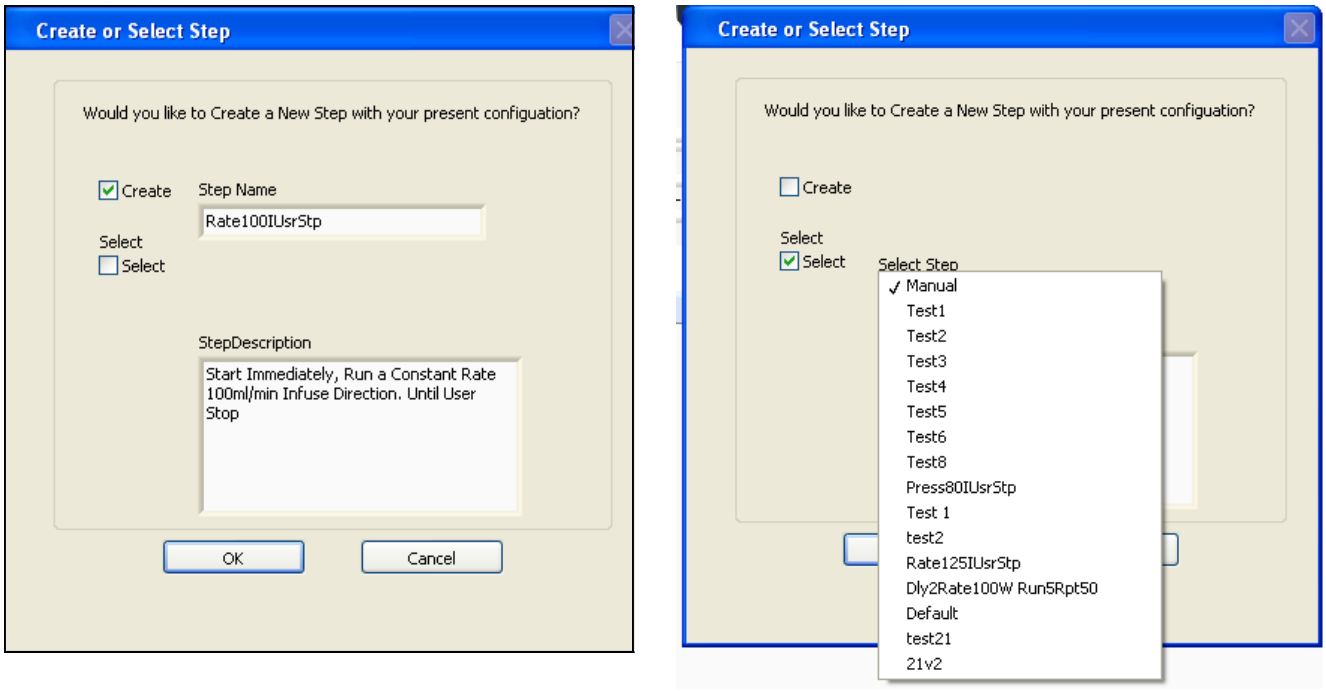


This screen gives the user the option to have the run stop automatically after a specified amount of time has elapsed. The default setting is **Stop Never**, which continues the run until the user manually terminates it.

Repeat Mode

On this screen, the user can choose between **No Repeat, Repeat Till (User Stop), Repeat Number of Times**, and **Repeat / Increase n Times**.

Step Creation

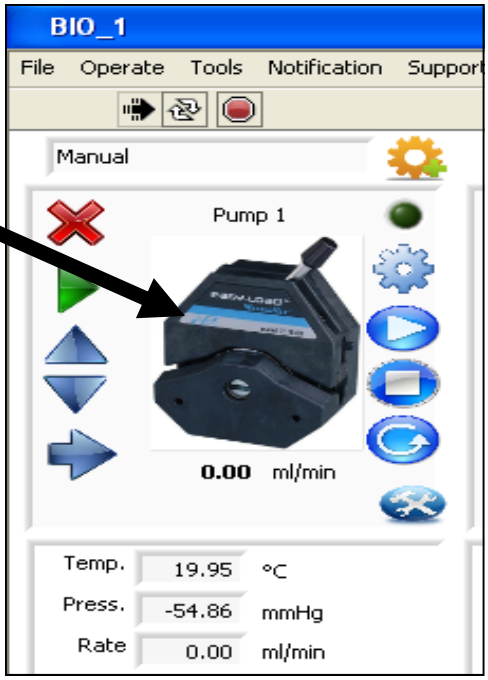


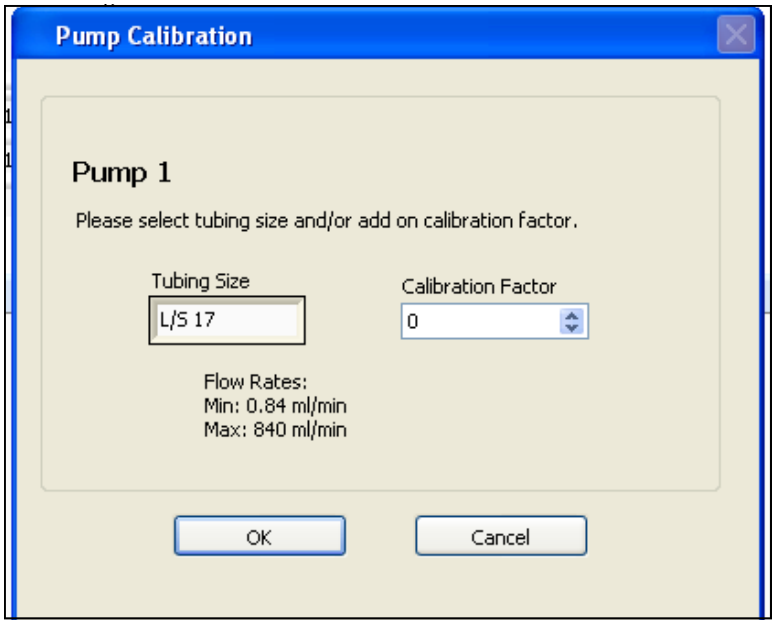
This window allows for the creation of a New Step or the selection of a previously used one.

If the create box is checked, a New Step can be created, with input boxes for the Step Name and a brief description. If the select box is checked, the user may select from the drop-down menu any of the previously created presets.

Pump Calibration

To access the pump calibration menu, click on the image of the pump that you wish to calibrate. From this menu, the correct tubing size can be selected and the calibration factor can be adjusted.





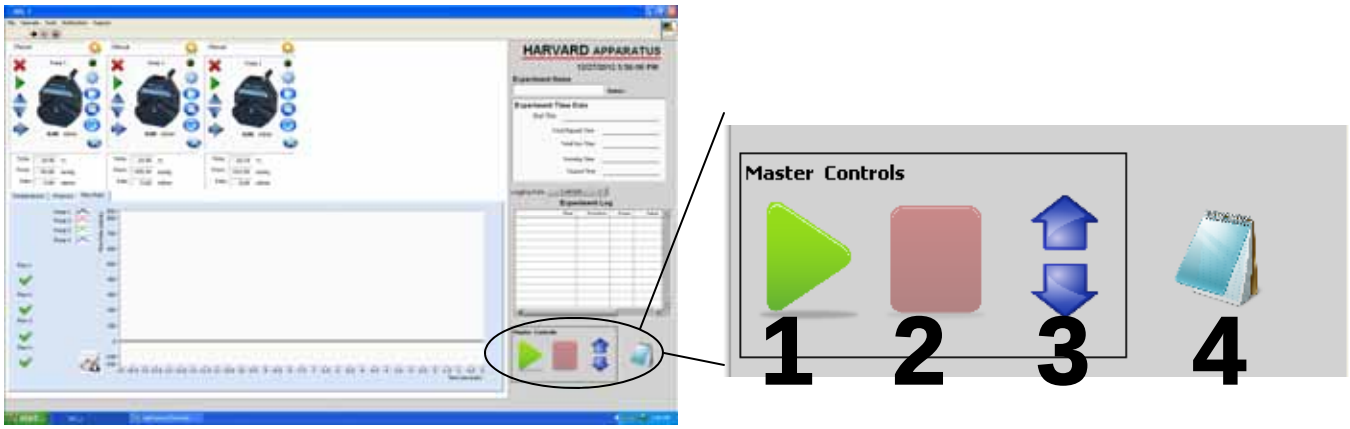
The calibration factor is selected based on the deviation from optimal flow rate.

In practice, the user may run at a specified rate into a graduated cylinder in order to test the actual volume delivered. If the volume delivered is less than the expected amount, the calibration factor can be set at a value greater than 1 to compensate for this deviation. Likewise, it can be set at a value 0<x<1 to account for positive deviation.

Flow Rate Constraints

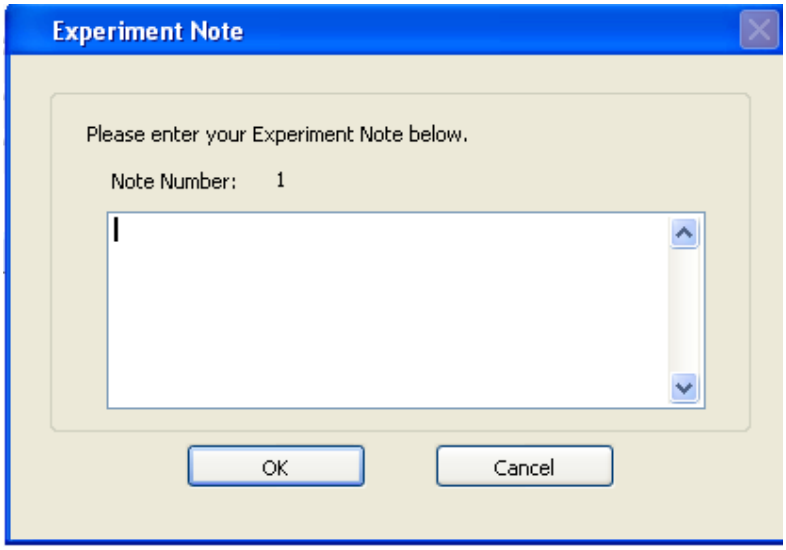
Tubing Size	Flow Rate Minimum (ml/min)	Flow Rate Maximum (ml/min)
L/S 13	0.018	18
L/S 14	0.63	63
L/S 16	0.24	240
L/S 25	0.51	510
L/S 17	0.84	840
L/S 18	1.14	1140

Master Controls



1) starts all pumps
2) stops all pumps
3) incrementally adjusts flow rate of all pumps
4) notepad

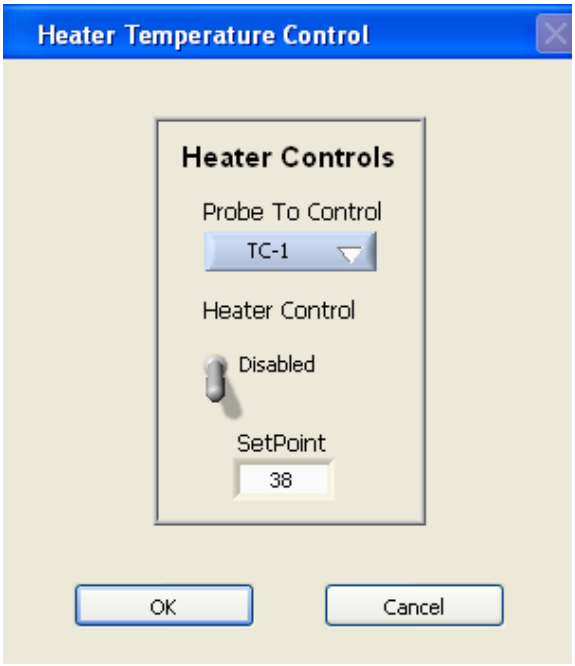
4



Any experiment notes entered will appear on the Experiment Log.

Note: All actions will be logged in the Experiment Log once the **Master Start** button is clicked. Any experimental changes will continue to be logged until the **Master Stop** icon is clicked. This is to prevent logging extraneous information while retaining valuable information about adjustments

Heater Temperature Control & Pressure Channel Calibration

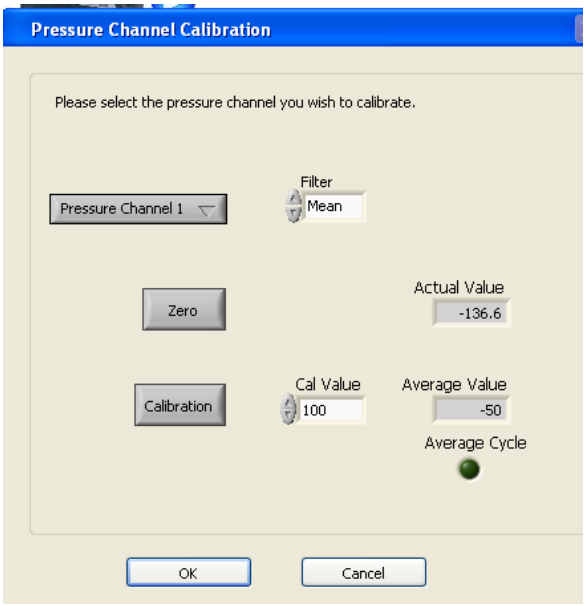


Access the Temperature Control menu through **Tools—>Heater**.

Probe to Control allows the user to select between four different probes, labeled TC-1 to TC-4.

Heater Control can be set to either Enabled or Disabled

Set Point allows the user to set the temperature in degrees Celsius.



The Pressure Channel Calibration menu can be accessed through **Tools—>Calibration—>Pressure**.

First select the desired **Pressure Channel**.

The **Filter** may be set to either Mean or Raw.

The **Actual Value** is the instantaneous value for the pressure reading.

Average Value is the average reading over a few seconds. The Average Cycle serves as an “update light” to let the user know when the average has been updated.

Use the **Zero** button to zero the actual value reading, and **Cal Value** along with the **Calibration** button to calibrate pressure readings.

Decellularization

Recellularization

Chapter 5: Care & Maintenance

Cross-Infection Prevention, Biohazardous Waste, and Product Disposal

Cross-Infection Prevention/Universal Precautions

All blood products or products potentially contaminated by blood or other body/animal fluids should be treated as potentially infectious materials. Personal protective equipment should be worn at all times when using the In-Breath Bioreactor to protect personnel from becoming contaminated as well as to help prevent cross-infection and cross-contamination.

Bench tops, equipment, and other potentially contaminated surfaces should be cleaned and disinfected according to the manufacturers' and/or the facility's procedures. Any article used to clean potentially contaminated surfaces should be disposed of as Biohazardous Waste.

CAUTION:

Failure to use the manufacturers' cleaning and disinfecting procedure could result in damage to the surface or equipment.

Biohazardous Waste

Dispose of biohazardous waste according to local Regulatory requirements.

Accessories

Peristaltic pumps with isolated heads capable of being used in a CO_2 incubator.

Cleaning

Flush all fluid paths with deionized water and either follow with or wipe with 70% IPA.

Stainless steel parts may be sonicated.

Parts List

Amount	Equipment	Part Number	
	Clamp, 1/8” (3.18mm)	xxxxxx	☐
	Clamp, 1/4” (6.35mm)	721670	☐
	Clamp, 3/8” (9.53mm)	721671	☐
	Connector, 1/8” (3.18mm)	xxxxxx	☐
	Connector, 3/8” (9.53mm)	721577	☐
	PharMed tubing, L/S 16 (0.12”, 3.1mm ID)	881105	☐
	PharMed tubing, L/S 17 (0.25”, 6.4mm ID)	881100	☐
	PharMed tubing, L/S 18 (0.31”, 7.9mm ID)	881101	☐
	PharMed tubing, L/S 25 (0.19”, 4.8mm ID)	881102	☐
	Teflon tubing, 1/8” (3.18mm) OD	xxxxxx	☐
	Teflon tubing, 1/4” (6.35mm) OD	30345	☐
	Teflon tubing, 3/8” (9.53mm) OD	30346	☐
	Teflon tubing, 1/2” (12.7mm) OD	30347	☐
	Tygon tubing, 1/8” (3.18mm) ID	721259	☐
	Tygon tubing, 1/4” (6.35mm) ID	721256	☐
	Tygon tubing, 3/8” (9.53mm) ID	721264	☐
	Tygon tubing, 1/2” (12.7mm) ID	721268	☐
	Y Barb, 3/8” (9.53mm)	721588	☐
	3/8” to 1/8” T fitting	xxxxxx	☐
			☐
			☐

Appendix A: PoleStar Setup and Operation

Application Instructions

Polestar Fluorescence-based Optical Biosensors

Harvard Apparatus

Version 1.21 061112

Written primarily for applications involving isolated organ and tissue. See manufacturer's manual for details regarding use in standard applications. This guide is NOT a replacement for the manufacturer's manual.

Table Of Contents

1 Introduction, Manufacturer's details

2 Safety Note

3 General Description, Application

3.1 Measurement Specifications

4 Initial Setup of DSP4000 Unit/Quick-start Guide

4.1 Installing the USB onto Computer

4.2 Connecting RTD Probes to Sensors

4.3 Integrating Sensors into System Setup

4.4 Connecting DSP4000 to Data Acquisition System

5 Calibrating Sensors, General

5.1 Set Barometric Pressure

5.2 Units Conversion, Data Acquisition 2-Point Calibration

5.3 Oxygen Sensor Calibration

5.4 Carbon Dioxide Sensor Calibration

5.5 pH Sensor Calibration

6 Viewing Data on DSP4000

6.1 Channel Readings – O2, CO2, pH Parameters

6.2 Channel Readings – Temperature and Pressure Readings

6.3 Channel Readings – ACG Readings

7 Changing expired sensors

7.1 Updating Lot Number for New Sensor Calibration

8 Cleaning and Maintenance

9 Troubleshooting Guide/ Ordering Information

1 Introduction, Supplier's detail

These Operating Instructions describe the function and use of the DSP4000 Optical Process Monitor. This manual is to be considered part of the system for its proper use and operation and should be kept close to the system at all times. The information provided on the DSP4000 has to be read in conjunction with the remaining system documentation for the application to which it is being put to use.

All the information contained in these instructions has been drawn up after careful examination but does not represent a warranty of product properties. Alterations in line with technical progress are reserved.

This DSP4000 unit is manufactured by:
POLESTAR TECHNOLOGIES and supplied to you by HARVARD APPARATUS. All inquiries regarding this system addition/application should be directed to Harvard Apparatus or to the Harvard Apparatus Authorized Distributor from whom this unit was purchased.

Harvard Apparatus
84 October Hill Rd
Holliston, MA 01746
Phone (United States) : 800-272-2775
Phone (others) : 508-893-8999
email: techsupport@harvardapparatus.com

Harvard Apparatus has locations in Canada, United Kingdom, Germany, Spain, and France.

2 Safety Note

Operation in hazardous areas and/or a flammable atmosphere is not permitted.

3 General Description, Application

The DSP4000 Optical Process Monitor can be configured for 1, 2, 3, or 4 Channels (defined by the number of LED modules present in the system). Units are identical in functionality

though the total sensor capacity may differ. In the descriptions below all configurations will be referred to as the "DSP4000."

The DSP4000 is an Optical Process Monitor designed to emit and sense light in a specific range of wavelengths for the purpose of determining the concentration of the presence of the parameter(s) for which the connected sensor(s) is (are) configured (pH, O2, CO2).

The sensors are supplied in a range of configurations to meet the various application specifications of the end users. Most frequently researchers who purchase the system from Harvard Apparatus will be utilizing flow-through sensors for use in one of the many perfusion systems available; however, in vivo applications are also common in which a puncture probe may be used. General purpose probes can also be used and immersed in any fluid. O2 and CO2 sensors can operate with fluid or with gas and therefore a wide variety of applications is possible.

The DSP4000 can be configured for 1, 2, 3, or 4 channels for one, two or all three of the parameters currently available (pH, O2, CO2). A dual channel system can be configured for measurement of 2 of the same parameters (requiring only one purchase of the firmware for that measurement) or 2 different parameters (as long as the firmware for the parameters of interest are both purchased) or 3 different parameters, with only 2 of the 3 sensors connected at any one time. This manual supplement is primarily designed to guide researchers utilizing the system for isolated perfused organ and tissue applications or who will be calibrating their sensors in the ranges outlined in section 3.1 of this document.

3.1 Measurement Specifications

Oxygen

Measurement ranges available:

High: 0-500% Air Sat

Low: 0-20% Air Sat

Units of Measurement available:

O2 %

O2 % Air Sat

O2 PPM (or PPB, or SUB-PPB)

O2 mmHg

O2 deg

pH

Measurement ranges available:

Low Physiologic: 4 - 7

Mid Physiologic: 5.5 – 10

Carbon Dioxide

Measurement range:

0 – 30 %

Units of measurement available:

CO2 %

CO2 mmHg

SMR

4 Initial Setup of DSP4000 Unit/Quick-start Guide

Please refer to the Quick Start Guide that was supplied with your DSP4000 unit. It is especially important to do the following upon initial setup:

* Open the door to the unit (loosen screws with fingers or screwdriver) and do the following:

    - Remove the USB Key and plug it into your PC and copy the contents to your Desktop (or preferred location on your computer). Take special note of the manual (recommended to print a copy if desired). Return the USB Key to the Unit

    - Verify that the toggle switch (next to USB KEY) is in the "OFF" Position before plugging in the AC Power Adapter (See Figure 2-3 of DSP4000 Optical Process Monitor User's Manual)

* Turn "ON" the DSP Monitor by flipping the toggle switch. The Monitor should warm up for 30 minutes before first time use and at any time when powering on from a full shut down (where the toggle switch inside the unit is in the "off" position). Generally the unit can be left in Standby Mode (the Main Menu is displayed) between uses unless one of the following requires a full shut down:

    • You are connecting I/O as described in Section 4 ("Electrical Connections") in the DSP4000 User's Guide

    • You need to upgrade the operating system to a new version. The DSP4000 will prompt you to upgrade the software when you restart the system (see Figure 2-12)

    • There will be an extended period between uses

* Refer to Quick Start Guide for connection of Fiber Optic Cables. NOTE: You must align the small tab on the ST Connector on your sensor with the notch in the Fiber Optic cable or the fitting will not connect.

* Recommended not to re-screw lid until your setup is complete and the unit is fully calibrated and ready to use

* **NEVER use a Power Strip to power on and off your DSP Unit**. This results in a "fuzzy" power cutoff that interferes with the memory and proper functioning of the unit.

* Proper methods for powering down the DSP4000:

- Go to Main Menu and select "Turn Off DSP4000." This allows you to put the unit into standby and avoid having to wait for a 60 minute warm-up period at the next use

- If you wish for a full power shutoff, then after the above step, open the DSP4000 and flip the toggle switch to "OFF."

* Saving the Log file is optional. If you have performed a calibration procedure or changed any other setting, or recorded measurement data onto the log file, it is advisable to save the log file.

4.1 USB Key

* It is important to identify which sensor was assigned to each channel in order to match the sensor/fiber optic cable and channel with the correct parameter, units and calibration data that were used during testing. Instructions on how to do this are described in section 2.4 of the DSP4000 Optical Process Monitor User's Manual. You can change the assignments later by loading each sensor's calibration file to a different channel, as discussed in Chapter 3, "Managing Configuration Files."

* Removing the USB Key while unit remains under power (without powering off the DSP4000):

For full instructions please refer to DSP4000 Optical Process Monitor User's Manual section 2.8. This is done when you wish to review the log file or update lot #/calibration information (such as

when a new sensor is purchased). Using this method also closes the current log file such that when measurement resumes, you will be recording onto a new log file. **note this does not work with older USB Keys where the software may freeze

4.2 Connecting RTD (Temperature) Probes to DSP4000 and Sensors

If your RTD Probe(s) were not purchased with the system and are purchased as an add-on, they will need to be wired into the DSP4000. (Additional information can be found in Section 4.2 of the DSP4000 Optical Process Monitor User's Manual.)

Step 1: Remove the three-pronged connector from the end of the RTD Probe (using a very small screwdriver) and carefully unwind the bare wire terminations and straighten them out

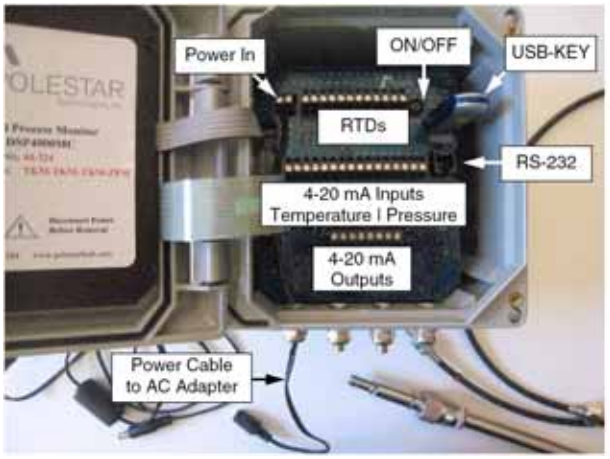
Step 2: Power off the DSP4000 unit by turning it off in the menu and then with the toggle switch inside the case. Unplug the unit from the AC Adapter.

Step 3: Using a small flathead screwdriver loosen the metal clamps for the applicable RTD inputs.

Step 4: The four cord grips on the bottom panel enable you to bring connecting wires into the DSP4000 enclosure while maintaining a water-tight seal. To bring the wire into the DSP4000 enclosure:

- Loosen the locking nut on the cord grip and remove the cylindrical metal plug. Figure 4-2 shows a DSP4000 where the left-most and right-most cord grips have already been used for the power cable and an RS-232 connection, respectively. The plug has been removed from the grip next to the RS-232 cable.
- Store the cylindrical plug in a safe place.

Channel	Terminal Labels		
Channel 1	RTD1_Black [1]	RTD1_Red [2]	RTD1_White [3]
Channel 2	RTD2_Black [4]	RTD2_Red [5]	RTD2_White [6]
Channel 3	RTD3_Black [7]	RTD3_Red [8]	RTD3_White [9]
Channel 4	RTD4_Black [10]	RTD4_Red [11]	RTD4_White [12]



Step 5: Insert the bare wires as per the following table. Clamp down using screwdriver

Connect the threaded RTD probe tip into your sensor. The Flowcells and the Tri-fold Flowcells

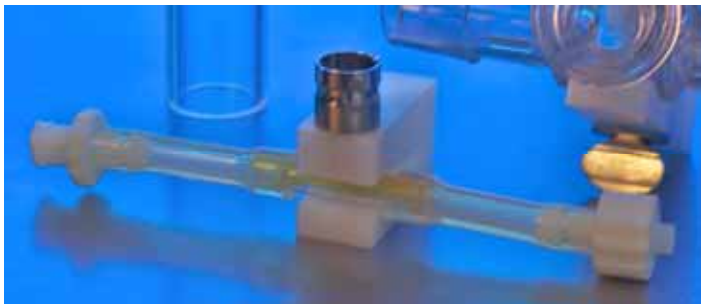
both have built-in threaded ports to accommodate the RTD probe.

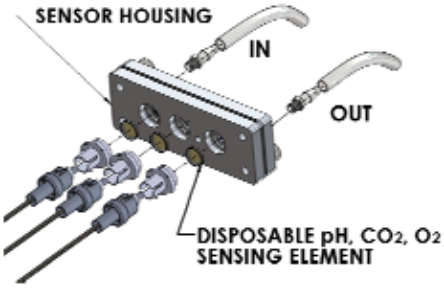
4.3 Integrating Sensors into System Setup

The Flow cells in the cell mounts have male and female Luer terminations.

Make sure that you always use a non-gas permeable or low gas-permeable tubing such as Tygon R-3603 or Tygon S-50-HL. Using a Luer-Barb connector kit, you can fit multiple barb sizes to the Luer terminations or connect these to each other end-to-end (for multiple sensor types).

The Tri-fold Flowcell has four threaded holes set in a square configuration (0.20" deep, 10-32 Thread/M5 Thread) to accommodate multiple mounting options. It can also be placed on the lab bench or clamped using rod/clamp system to the relevant portion of the perfusion system.





4.4 Connecting DSP4000 to Data Acquisition System

• If you purchased a DAQ-DSP4000 Interface Cable, this has come preinstalled and configured to work with a standard 0-10V Analog to Digital Data Acquisition System (such as a PowerLab, Data, NI-DAQ, iWorx, Biopac, or other). These cables terminate in a Male BNC connector. Utilizing DAQ hardware that has analog inputs other than female BNC requires the use of an adapter to your input type or removal of the BNC connector to bare leads.

• Connect the BNC output cables to the Analog Input BNC connectors on your Data Acquisition System. Please connect to the channel on which you wish the particular reading to be recorded.

- The output range of the analog output is as follows:

Sensor Parameter	Units	4 mA =	20 mA =
O_2 High Level	mmHg	0 mmHg	5000 mmHg
	%	0%	100%
	% Air Sat	0% Saturation	100% Saturation
	PPM	0 PPM	5000 PPM
	Deg	-180	180
O_2 Medium Level	PPB Dissolved	0 PPB	5000 PPB
	PPM Gas Phase	0 PPM	5000 PPM
	Deg	-180	180
O_2 Low Level	PPB Dissolved	0 PPB	5000 PPB
	PPM Gas Phase	0 PPM	5000 PPM
	Deg	-180	180

- Minimum measurement (4mA) is 2V output

pH	pH	Scale read for the channel from DSPCALIB.DAT in the USB-KEY (Factor 2 and Factor 3 for channels 2 and 4 in Figure 4-9)	
	SMR	0	3
CO_2	mmHg	0 mmHg	High scale value read for the channel from DSPCALIB.DAT in the USB-KEY (Factor 1 for channel 3 in Figure 4-9)
	%	0%	
	SMR	0	3

- Maximum measurement (20mA) is 9.9V

5 Calibrating Sensors, General

Calibration gas and solution:

The DSP4000 typically only requires a 1-point calibration at a value near the range in which you will be measuring:

- pH measurements, use a buffer standard of 8.0.
- When a 95% O2/5% CO2 gas mix is the gas used for aeration and pH maintenance of your perfusion buffer, this gas can be used for the 1 point calibrations of both Oxygen and Carbon Dioxide (gas-saturate a non-bicarbonate buffered solution). If your perfusion system requires buffers to be deoxygenated (such as in lung applications) or use buffer equilibrated with room air, then air-saturated solution (non-bicarbonate buffered) should be used for calibration. In these situations it is common to use some 5% CO2 gas mix for the 1 point calibration of the CO2 sensor (such as 5% CO2) with balance air, or 95% N2/5% CO2.

***TECHNICAL NOTE*: Oxygen Sensor calibration is always performed based on air saturation and not dissolved oxygen saturation. 20.9% DO = 100% Air Saturation. 95% DO = 454% Air Saturation**

If a 2 point calibration needs to be performed, then the following zero/low standards should be used:

pH: buffer pH 5 or lower (this is the minimum output of the Polestar)

CO2: Room Air-equilibrated distilled water, which has a CO2 level of 0% or Nitrogen Gas

O2: pO2 zero solution (Catalog Number 73-3812) or Nitrogen gas saturated solution

The optical sensing films consist of fluorescent indicator chemistries immobilized in either hydrophilic (pH sensor) or hydrophobic (DO and CO2 sensors) polymer membranes. The gaseous (O2/CO2) or ionic (H+) species of interest move freely between the solution and interior portions of the sensing membranes to maintain an equilibrium of partial pressure (O2, CO2) or chemical potential (pH). The time to reestablish the equilibrium condition following a change in the solution is dependent upon the species rate of diffusion through the membrane (faster at 37°C). For the purpose of calibration, it is recommended that the user allow a minimum of 10 minutes exposure of cycling the calibration solution through the sensor to ensure complete equilibration of the sensing film. Note: it is important that the calibration solution flow over the sensor during the calibration process. Failure to use a flowing calibration solution can affect the calibration results

(flow through a flow-through sensor or use a magnetic stirrer).

If calibrating with gas, the gas should flow through the sensor for at least 5 minutes.

Application Note: If you wish to scale the OUTPUT of the DSP4000 for higher resolution within a smaller range of the total range of the sensor, use the DSP Configuration Utility (see section 4.6 "Scaling Values in the Calibration File" of the DSP4000 Optical Process Monitor User's Manual).

Temperature Influence on CO2 and O2 Measurements:

For accurate calibrations and measurements, the temperature needs to be known and actively compensated. You should have the temperature either manually set at the temperature at which the calibration is taking place (you can put a small temperature probe into the line to measure) or you can use an RTD probe that is wired into the DSP4000 unit. The RTD probe is the preferred method as you will have continuous temperature compensation and therefore will not need to change any temperature settings on the DSP4000 between calibration and measurement.

The DSP4000 can be configured such that a single RTD probe can provide the continuous temperature source for all channels. This is appropriate for a sensor like the Tri-fold Flow Cell. If sensors are located in different areas, then multiple RTD probes can be used. A Channel is instructed to look at either a Common Source RTD (for example, you can designate the RTD Probe on Channel 1 to be the Common Source and the other temperature-dependent sensor on the Tri-Fold Flow cell can be directed to use the Common Source). If another individual sensor is located elsewhere, then a second RTD probe can be installed on that channel and you would then direct the DSP4000 to use "RTD" as the source for that Channel.

If an RTD probe is not used, then the temperature can be set manually.

.

Manual Temperature Input

Manual Input allows you to specify the temperature value to use for compensation. This option should only be considered for applications where there is little or no variation in temperature or where the temperature is well regulated.

To set the temperature manually:

1. Select **Manual Input** in the Temperature Source menu and press ENTER to show the currently set temperature for manual input. The default is 20° C. The range is -20° C to 100° C.

2. Press the up or down arrow to increase or decrease the setting by 0.1° C, or hold the key down to rapidly change the value. Press the left or right arrow keys to change the position of the cursor to the tens, ones, or tenths position.

3. Press ENTER to accept the displayed setting and return to the Setup menu.

5.1 Set Barometric Pressure

Measurements taken for CO_2 and O_2 are sensitive to barometric pressure. Unless a pressure monitor is available with a 4-20mA analog output (which can be fed into the DSP unit), an accurate barometric pressure should be read from a NIST certified barometer and manually entered into the unit on a daily basis. Instructions for manual input of pressure are found in section 5.6.1 of the DSP4000 Optical Process Monitor User's Manual.

Options for **continuous barometric pressure compensation** are:

RM Young 61302L Barometer with 4-20mA analog output standard

Vaisala BAROCAP® Digital Barometer PTB330

Manual Pressure Input

You can manually enter a pressure value in units of mmHg or PSI. Input values must be absolute pressure, that is, barometric pressure plus gauge pressure. The default value is 14.7 PSI (760 mmHg). To use a manually input value for pressure compensation:

1. Select Pressure from the Setup menu and press ENTER to display the current setting, and then press ENTER to show the pressure input options.

2. Select one of the manual input options and press ENTER to show the currently specified input.

3. Press the up or down arrow to increase or decrease the setting one unit at a time, or hold down the key to rapidly change the value.

4. Press ENTER to accept the displayed value and return to the Setup menu.

Calibration Menu on DSP4000

To reach the calibration menu, select "Setup Mode" from the Main Screen. Choose the Channel being calibrated and press ENTER. Then scroll down to Calibration, press ENTER and follow instructions.

5.2 Units Conversion, Data Acquisition 2-Point Calibration

After the DSP4000 Optical Process Monitor has undergone the 1-point calibration(s) for the channel(s) installed, you may proceed with applying the units conversion/calibration to your data acquisition system software.

For units conversion in LabChart, follow these steps:

1. Connect the Analog Output Cables to the Powerlab
2. Ensure that the Range is set at 10V on the Input Amplifier. Set up the channel appropriately in the Channel Settings Window or individually on each channel as per LabChart instructions
3. Press Start and record the upper calibration value that was used for the 1-point calibration. Once the reading is stable and you have your sample captured, proceed to step 4. Make note of the reading on the DSP4000 unit during this trace recording as this will be the value used in your units conversion.
4. Apply the low/zero conditions to the flowcell. For example, for oxygen this may be the upper calibration point of 95% O2 followed by buffer that has been aerated for 30 minutes or which is equilibrated with the aeration gas. Continue recording until the stable phase is reached and you have a suitable trace for calibration. Make note of the reading on the DSP4000 unit during this trace recording as this will be the value used in your units conversion.
5. Highlight the full calibration data trace (high and low point) for one sensor type at a time and choose “Units Conversion” from the right-click drop down for that channel. Follow normal calibration procedure using the values obtained from the DSP for the high and low points.

5.3 Oxygen Sensor Calibration

It is recommended to use the 1-point calibration on the DSP4000. When complete, put the DSP4000 into RUN MODE and collect data into LabChart. Switch to the lower oxygen solution (such as aerated buffer), to capture the low value. Once done, stop data recording and highlight the full section of data that includes both the high and low calibration points. Right click on the range in the channel and choose “Units Conversion” from the drop-down menu. Use the values displayed on the DSP4000 for the 2 point calibration in LabChart.

5.4 Carbon Dioxide Sensor Calibration

A 1-point calibration should be performed on the DSP4000 when a known gas concentration, e.g. 5%, is not read accurately on the DSP Display. Typically this calibration point is at 5% as that is the concentration of CO2 that is used to maintain proper pH in most bicarbonate buffers used in perfusion systems. When the 1-point calibration is complete, put the DSP4000 into RUN MODE and collect data into LabChart. Switch to the zero gas or solution for 5 or 10 minutes (respectively) (or until the reading has fully stabilized) and continue to collect data for the low point. Once done, stop data recording and highlight the full section of data that includes both the high and low calibration points. Right click on the range in the channel and choose “Units Conversion” from the drop-down menu and use the values that are displayed on the DSP4000 for the 2 point calibration in LabChart.

5.5 pH Sensor Calibration

When the pH reading on the DSP4000 is not accurate, a 1-point calibration can be performed on the DSP4000. A calibration buffer of a value near the pKa of the sensor is used. For a 5.5-10 pH sensor, the pKa, or midpoint of the response curve falls, at 8.2, therefore we choose pH 8 as the preferred calibration buffer for this sensor. When complete, put the DSP4000 into RUN MODE and collect data into LabChart. Switch to pH 6 or other lower pH solution that falls below your optimal area of interest. Calibration Solution for 10 minutes (or until the reading has fully stabilized) and continue to collect data. Once done, stop data recording and highlight the full section of data that includes both the high and low calibration points. Right click on the range in the channel and choose “Units Conversion” from the drop-down menu and use the values that are displayed on the DSP4000 for the 2 point calibration in LabChart.

6 Viewing Data on DSP4000

Run Mode will by default show you the channel measurement data for the sensor plugged into that channel. The AUX button changes the display to give current readings of other channel-specific information:

If the AUX button is pressed, it scrolls the screen through displays of temperature/pressure, AGC Value, and Channel Measurements.

6.1 Channel Readings – O2, CO2, pH Parameters

These are displayed as in the image to the left. The channel designation followed by the numerical value and then the units that have been assigned to that channel.

This display is showing a three channel DSP4000 with two channels disabled.

6.2 Channel Readings – Temperature and Pressure Readings

Pressing the AUX key will give you a display that reads the channel designation followed by the temperature/pressure values per channel. These will only show real time data from an actively compensating input source (barometer or RTD probe).

6.3 Channel Readings – AGC Readings

These readings can be useful for feedback regarding sensor effectiveness or placement in regards to the fiber optic cable. High AGC readings indicate a high gain applied to the LED which can be caused by photo-bleaching of the sensor chemistry or by improper alignment of the chemistry in relation to the fiber optic cable.

7 Changing expired sensors

The Status LED in the upper right corner of the DSP4000 front panel provides a visual indication of the AGC or RAGC level during operation. The Status LED is:

- **Green** when the AGC level is less than 3000 for all four channels
- **Yellow** when the level for least one channel is between 3000 and 3500
- **Red** when the AGC for one channel exceeds 3500. An AGC or RAGC reading of 3500 or higher indicates a need to replace the sensing element, or an obstruction or break in the optical path.

When the Status light is **red**:

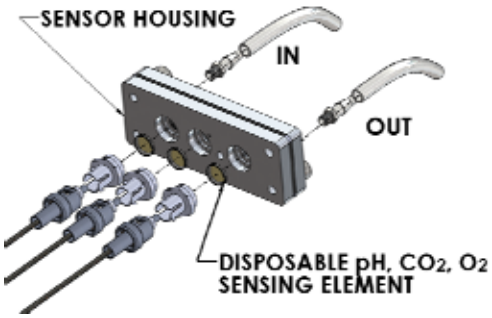
1. Check the AGC level of each channel to identify the channel causing the Status LED alert. See "AGC Display" Section 5.8 of the DSP4000 Optical Process Monitor User's Manual for information on accessing the display from the Setup menu.
2. Inspect the BioProbe connections and the optics on the problem channel to determine whether the high AGC is caused by an obstruction or break in the optic path. See "Inspection Procedures" in Section 6.1.1 of the DSP4000 Optical Process Monitor User's Manual.
3. Replace the sensing element if the AGC is still high after resolving any problems with the fiber optic path.

Flow-cells (glass insert with chemistry painted internally):

1. Remove the glass insert by loosening the screw with an allen/hex wrench and sliding the flow cell out.
2. Contact Harvard Apparatus or your Harvard Apparatus Appointed Distributor for the correct re placement part number for the flow cell insert for your measurement (i.e. O2 PPB, O2 PPM, O2 Sub-PPB, CO2, pH 5.5 – 10 or 4 - 7).

Tri-Fold sensor:

1. Remove the Fiber Optic cable(s).
2. Using a wrench (if unable to remove with fingers), unscrew the ST Connector on the channel that needs a replacement sensing disk.
3. Contact Harvard Apparatus or your Harvard Apparatus Appointed Distributor for the correct re-placement part number for your sensor type (*i.e.* O_2 PPB, O_2 PPM, O_2 Sub-PPB, CO_2, pH 5.5 – 10 or 4 - 7).



Puncture probe:

1. Remove the puncture probe from the fiber optic cable. This sensor is purchased as in the catalog as it has no replacement portions as it is a disposable item.
2. Update the configuration file on the USB-KEY, as described in "Loading the File onto the USB-KEY" Section 3.4 of the DSP4000 Optical Process Monitor User's Manual.

BioProbe:

1. Unscrew the tip from the BioProbe by twisting it counter-clockwise.

CAUTION: The sensing element is designed to seal when finger-tight. Never use pliers or any other tool to loosen or tighten the sensing element, as the tools can damage the sensing element and probe body.

2. Screw the replacement element onto the end of the probe. Calibration information for the sensing element is contained in a calibration file that includes the sensor type and the calibration factors for all elements in the same manufacturing lot. You can download the calibration file from the Support area on the Polestar Technologies web site.
3. Update the configuration file on the USB-KEY, as described in "Loading the File onto the USB-KEY" Section 3.4 of the DSP4000 Optical Process Monitor User's Manual.

7.1 Updating Lot Number for New Sensor Calibration

See Chapter 3 ("Managing Configuration Files) of the DSP4000 Optical Process Monitor User's Manual for using the Configuration Utility to assign new lot numbers to the measurement channel. The configuration utility is also used to reassign channels to different measurements.

8 Cleaning and Maintenance

Optical Sensors are not affected by ambient light when in use; however, they are sensitive to light and should be stored in the dark when not in use.

The sensors can be cleaned using MUCASOL, which is preferred when using the sensors in conjunction with a Hugo-Sachs Isolated Organ or Tissue Perfusion System whereby there are wetted components constructed from Plexiglas.

If the fiber optic cables have been detached and stored or if they are otherwise possible dirty you should use a piece of scotch tape to tap on the tip to remove particulate matter.

Additional in-depth maintenance information can be found in Chapter 6 “Care and Maintenance” of the DSP4000 Optical Process Monitor User’s Manual.

You will need the following items to inspect and maintain the DSP4000:

- De-ionized water
- Chem Wipe® or soft lint-free cloth
- Scotch tape
- Magnifying glass
- Compressed air or nitrogen
- Adjustable wrench.

All Polestar sensors (including sensing elements, iDots, peel and sticks, and puncture probes) are sensitive to ambient light and will photo-bleach over time unless stored in a dark place between uses. Bright sun light will accelerate the aging process of the sensor. Photo-bleaching affects the useful life of the sensing element, but not the accuracy of measurements.

Troubleshooting Guide

Symptom	Explanation of Problem	Action
The DSP4000 does not display measurements or menus.	Insufficient or too much power is being applied to the DSP4000.	1. Disconnect power from the DSP4000. 2. Verify that the voltage is 24 VDC and the current is at least 1.5 Amps. 3. Re-connect the power.
The DSP4000 powers up with a USB error.	The USB-KEY is not recognized.	1. Re-seat the USB-KEY. 2. Press **ENTER**.
The DSP4000 does not go into Run mode, or kicks out shortly after startup. The Status LED is red.	The AGC for at least one channel has reached 4000, the maximum value because: • There is a poor optical connection. • A sensor element is at the end of its life.	1. Verify all optical connections. 2. Check the AGC for each channel. 3. Replace sensing elements as needed.
The Status LED is yellow.	The AGC for at least one channel has reached 3000 because: • Dirt has accumulated on an the optical surface. • The sensing element is near the end of its useful life.	1. Check the AGC for each channel. 2. Clean the optical surfaces using Scotch tape as dust and dirt particles may result in signal loss. 3. Replace sensing elements as needed.

If none of the above apply and you are seeing “AGC Limit,” please check to make sure that the sensor chemistry is as close to the LED/fiber optic as possible. If using a glass flow-cell, check to make sure that it is seated in the cell mount such that the chemistry is between the light and the perfusate/media.

Ordering Information

88-0258	O_2 Replacement Kit (sensor, glass window & O-Ring)
88-0260	pH Replacement Kit (sensor, glass window & O-Ring)
88-0261	CO_2 Replacement Kit (sensor, glass window & O-Ring)
880258	O_2 Replacement sensor
880260	pH Replacement sensor
880261	CO_2 Replacement sensor
88-0263	REPLACEMENT QUARTZ 0_2 PPB SENSOR INSERT FOR SINGLE QUARTZ FLOWCELL MOUNT
72-8161	BUFFER SOLUTION pH 8.00, 500ml
72-8162	BUFFER SOLUTION pH 7.41, 500ml
72-8163	BUFFER SOLUTION pH 6.00, 500ml
72-8164	BUFFER SOLUTION KIT: pH 6, 7.41, 8, 500ml/EA
880194	Trifold Connections Kits 1/16”
880195	Trifold Connections Kits 1/8”
880196	Trifold Connections Kits 1/4”

Appendix B: Frequently Asked Questions

Hollow Organ Bioreactor

Is the retainer clamp missing from my unit?

The retainer clamp design did not fit as well as it was supposed to, and since it did not seem to be necessary, we have not been shipping it with the product. A new clamp is in the process of being developed to make sure the drive mechanism does not slip and disengage itself.

How does the "seal wash inlet" and outlet work (for hollow organ systems)?

This passage is designed to take a non-salt solution and flush it through a separate space between the two outside O-rings on the driveshaft. This will prevent any buildup of evaporated salt crystals from forming on the turning shaft during long periods of use. As an example, an intravenous drip bag with water set to between .1 and .5mL per minute to continuously flush the seal wash path could be used.

For the hollow organ bioreactor, should I sterilize using plasma sterilization or would autoclaving work?

The materials of construction are Teflon reservoir, stainless steel baseplate, driveshaft, and valves, silicone rubber O-rings, Kynar Luer fittings, polycarbonate cover, and PEEK arbor components. All of these materials will withstand steam sterilization.

One CAUTION, however…. **Please disassemble all the valve and driveshaft parts from the Teflon block before steam sterilization**. The high temperature may cause the machined holes in the Teflon to become deformed by the parts pressed into them. Reinstall the valves and driveshaft after the Teflon cools down.

The Kynar Luer fittings have been installed using silicone adhesive to hold them in place and prevent leaks. It should not be necessary to remove these fittings from the Teflon because of the sterilization process.

Can washing be done with [reagent]?

These materials of construction will withstand virtually all biological reagents and cold sterilization agents (such as Cidex, Cidex OPA, Mucasol, etc).

Appendix B: Frequently Asked Questions

Appendix B: Frequently Asked Questions

HARVARD
APPARATUS
REGENERATIVE TECHNOLOGY

Harvard Apparatus Regenerative Technology, 84 October Hill Drive, Holliston, MA 01746 USA
www.harvardbioscience.com ¨ 508.893.8999¨regenmedicine@harvardapparatus.com